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Chrysosporium linfenense: a new *Chrysosporium* species with keratinolytic activity

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Abstract — *Chrysosporium linfenense*, a new *Chrysosporium* species, was collected from Shanxi, China, described, and illustrated. Differences between *C. linfenense* and related species were analyzed based on the morphological and DNA sequence characters. Diagnostic characters of *C. linfenense* are conidia that are solitary or often in chains of 2–3, mostly ellipsoidal or fusiform, few clavate, and smooth-walled; intercalary conidia are absent. The presence of keratinase also suggests that *C. linfenense* possesses a keratinolytic activity.

Keywords — mitosporic fungi, morphology, molecular analysis, classification

Introduction

Chrysosporium species distributed around the world can produce many useful metabolites, especially keratinase, which can be used widely in the chemical industry and environmental protection, medical, and agricultural fields (Kushwaha 2000, Liang et al. 2007). The strain studied (GZUIFR-H31) was isolated from the rhizosphere soil of *Cedrus deodara*. Based on morphological and ITS1-5.8S-ITS2 rDNA sequence characters, this fungus was identified as a new species of *Chrysosporium, C. linfenense*.

Materials and methods

Sample collection and strain isolation

Strain GZUIFR-H31 was collected and isolated from the rhizosphere soil of *Cedrus deodara* in Linfen city, Shanxi Province, China. After a soil sample was mixed vigorously

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Table 1 Fungi used in the study with their GenBank accession numbers.

Names	GenBank No.	Name	GenBank No.
Amauroascus mutatus	AJ271565	*C. merdarium*	DQ888721
A. niger	AJ271563	*C. minutisporosum*	AJ131689
Aphanoascus hispanicus	AJ439438	*C. pilosum*	AJ390385
Ap. punsolae	AJ439440	*C. pseudomerdarium*	AJ390386
Ap. terreus	AJ439443	*C. queenslandicum*	AB219228
Auxarthron alboluteum	AB361630	*C. siglerae*	AJ131684
Castanedomyces australiensis	AJ131785	*C. submersum*	AJ131686
Chrysosporium articulatum	AJ007841	*C. sulfureum*	AJ390387
C. carmichaelii	AJ007842	*C. synchronum*	AJ390388
C. europae	AJ007843	*C. tropicum*	AJ131685
C. evolceanui	AJ005368	*C. undulatum*	AJ007845
C. filiforme	AJ131680	*C. vallenarense*	AJ390389
C. fluviale	AJ005367	*C. vespertilii*	AJ007846
C. georgiae	AJ007844	*C. zonatum*	AB219229
C. indicum	AJ005369	*Coccidioides immitis*	EF186784
C. keratinophilum	AJ131681	*Co. posadasii*	EF186786
C. linfenense	FJ392561	*Morchella conica*	AM269501
C. lobatum	AJ131688	*M. elata*	EF080996
C. lucknowense	AJ131682	*Uncinocarpus orissi*	AJ390393
C. mephiticum	AJ131683	*U. queenslandicus*	AB361646
C. merdarium	AJ390384		

with sterile water in a Erlenmeyer flask, the soil suspension was transferred to plates of Martin's medium and incubated at 30°C. Then the pure cultures were collected, transferred to PDA's slants, and stored at 4°C in the Institute of Fungus Resources, Guizhou University.

Strain identification and keratinolytic activity

The strain was transplanted to potato dextrose agar (PDA), incubated at 30°C for 14 days, and identified based on colony characters, conidiogenous structures, and keratinolytic activity (Oorschot 1980) and molecular analysis.

Keratinolytic activity was evaluated by the fungal capacity to degrade human hair on the surface of Czapek agar medium with carbon-free and nitrogen-free sources (Carmichael 1962). Keratinolytic evidence was examined by microscope.

DNA extraction and amplification

Taq enzyme and dNTP were from Shanghai Sangon. The strain GZUIFR-H31 was incubated on PDA and the fresh sporulating cultures were used for DNA extraction according to Tigano-Milani et al. (1995). The extracted DNA was stored at –20°C.

The internal transcribed spacer (ITS) region including the 5.8S rDNA was amplified by polymerase chain reaction (PCR) using the primers ITS5 (5'- GGT GAG AGATTT CTG TGC -3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). After a first denaturation step at 94°C for 5 min, the amplification reaction was performed for 35 cycles with denaturation at 94°C for 40 s, annealing at 49°C for 40 s, and extension at72°C for 1 min; followed by a final extension step at 72°C for 10 min. PCR products

were purified and sequenced with the above primers by Beijing Sunbiotech Co. Ltd. The sequence of ITS1-5.8S-ITS2 rDNA region of strain GZUIFR-H31 was submitted to GenBank (accession number: FJ392561).

Phylogenetic analysis

Strains listed in Table 1 were used in the DNA sequence analysis. Some ITS1-5.8S-ITS2 region nucleotide sequences of representative *Chrysosporium* species were obtained from GenBank. The sequences of *C. linfenense* and related fungi species were aligned using the Clustal X1.83 computer program for multiple sequence alignment and corrected manually. The phylogenetic tree was constructed by neighbor-joining method (NJ) of MEGA version 4.0 (Kumar et al. 2004). Confidence values for individual branches were determined by bootstrap analysis (1000 replications).

Results and discussion

Taxonomy

Chrysosporium linfenense Z.Q. Liang, J.D. Liang & Y.F. Han, **sp. nov.** Fig. 1

MycoBank MB512863; GenBank FJ392561

Conidia terminalia et lateralia ex hypha principali vel ramulis lateralibus oriunda, sessilia vel in brevibus protrusionibus,solitaria vel 2–3 catenata, hyaline vel subhyalina, laevia, ellipsoidea vel fusiformia, 3.2–5.4 × 1.4–2.2 µm, raro clavata, 4.2–6.5 × 1.6–2.5 µm, cum cicatricibus basilaribus 1–2 µm. Conidia intercalaria absentes. Chlamydosporae absentes. Ad 40°C non-crescunt. Species ceratinolytica. Reproductio sexualis non videtur.

***Holotypus**: GZUXIFR-H31, e solo, Linfen, Provincia Shanxi, VI, 2006; in Guizhou Univ., conservatur.*

Etymology: referring to the region from which the fungus was isolated.

Colonies incubated on PDA at 30°C reached 72mm diam after 14 days, white to cream, short fluffy to powdery, dense, slightly raised at centre; slightly loose in margin, not well defined, fimbriate; reverse white to light yellow. Hyphae hyaline, smooth- and thin-walled, 0.8–1.5(–2.3) µm wide; Racquet hyphae present, 4.5 µm wide, "racquet" 9 µm wide. Terminal and lateral conidia sessile or on short protrusions or lateral branches of variable length, solitary, often in chains of 2–3 or in cluster of 2, subhyaline, smooth, thin-walled, most ellipsoidal or fusiform, 3.2–5.4 × 1.4–2.2 µm, some clavate, 4.2–6.5× 1.6–2.5µm with basal scars measuring 1–2 µm, profusely sporulating. Intercalary conidia absent. Chlamydospores absent.

Teleomorph: unknown.

Growth Temperatures: minimum 15°C, optimum 30°C, maximum 40°C.

Keratinolytic Activity: Keratinolytic.

Distribution: Shanxi province, China

Material examined: The holotype GZUIFR-H31 was isolated from the rhizosphere soil of *Cedrus deodara* (Roxb.) G. Don. The paratypes, GZUIFR-H25, GZDXIFR-H26,

and GZUIFR-H29, were isolated respectively from rhizosphere soils of *Euonymus japonicus* Thunb., *Platanus orientalis* L., and *Chukrasia* sp., all obtained in Linfen city, Shanxi Province, China during June, 2006. All strains above are deposited in the Institute of Fungus Resources, Guizhou University.

Comments: *Chrysosporium linfenense* is characterized by a white colony, racquet hyphae, and conidia borne on slightly swollen conidiogenous cells. *C. indicum* (H.S. Randhawa & R.S. Sandhu) Garg 1966 and *C. minutisporosum* P. Vidal & Guarro 2002 also have racquet hyphae and swollen conidiogenous cells, but *C. indicum* often has obovoid to ellipsoid or cymbiform conidia with slightly echinulate walls, and *C. minutisporosum* conidia have verrucose walls and are pyriform, subglobose, or clavate. Additionally, both *C. indicum* and *C. minutisporosum* occasionally form intercalary conidia (Oorschot 1980, Vidal et al. 2002). *Chrysosporium fluviale* P. Vidal & Guarro 2000 is differentiated from *C. linfenense* by obovate, clavate, nearly ellipsoid or pyriform conidia with minute warts and rare intercalary conidia (Vidal et al. 2000) (Table 2). Additionally, lateral conidia of *C. linfenense* often form chains of 2–3. *Chrysosporium linfenense* is diagnosed by conidia that are solitary or often in chains of 2–3, mostly ellipsoidal or fusiform (a few clavate), and smooth-walled and by a lack of intercalary conidia.

Table 2 A comparison of morphological characters in *C. linfenense* and its related species.

Names	Conidial characters			
	Shape	Size (μm)	Wall	Intercalary conidia
C. fluviale	Obovate, clavate, nearly ellipsoid or pyriform	(3.5–)4–6.5(–15) × (1–)2–3(–3.5)	Regularly minutely warty	Very rare
C. indicum	Obovoid to ellipsoid, often cymbiform	3.5–7.5 × 1.5–3	Smooth to sl. echinulate	Infrequent
C. linfenense	Most ellipsoidal or fusiform, also clavate	3.2–5.4 × 1.4–2.2	Smooth	Absent
C. minutisporosum	Pyriform or subglobose, also clavate	3–4(–11) × (1.5–)2–2.5(–3.5)	Verrucose	Very rare

Molecular identification

A BLAST search in GenBank was performed using the *C. linfenense* ITS sequence as the query. Close matches showing maximal sequence identities of 80–97% included *Chrysosporium* spp. and other related species. The ITS sequences of these species were retrieved from GenBank for phylogenetic analysis. Relationships of *C. linfenense* and related species were showed in the phylogenetic tree based on analysis of rDNA ITS1-5.8S-ITS2 sequences (Fig. 2). *Morchella conica* Pers. 1818 and *Morchella elata* Fr. 1822 were designated outgroups.

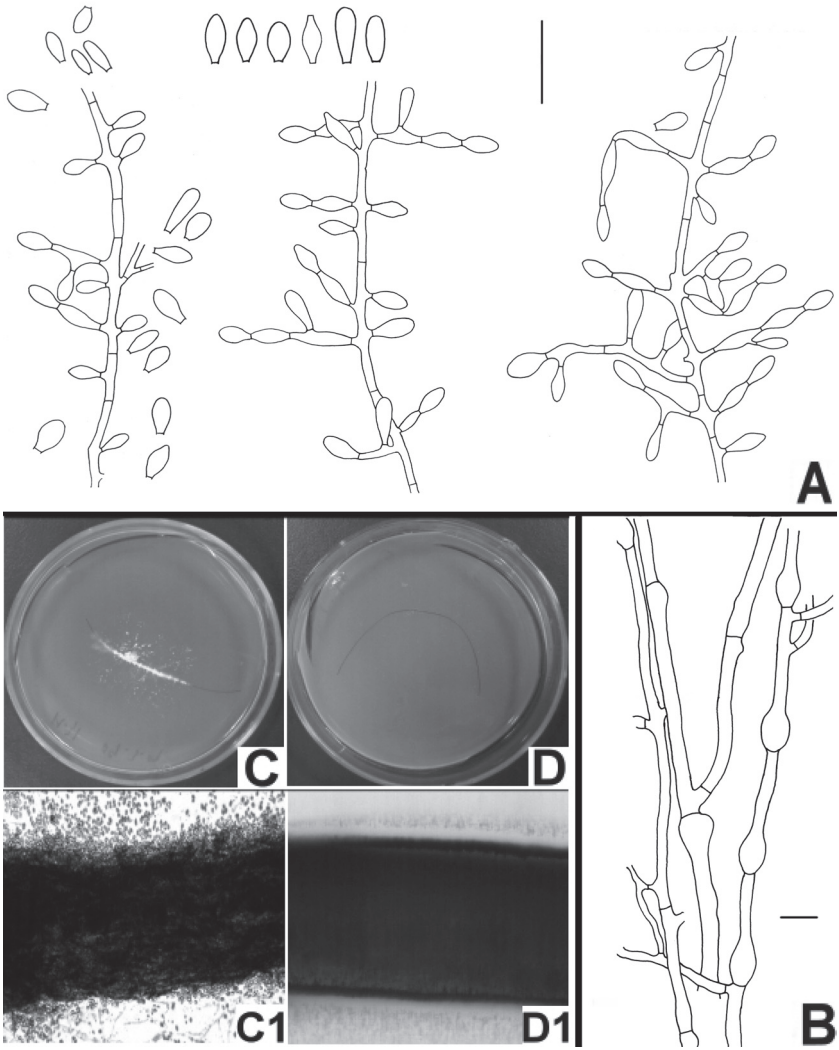

Fig. 1 *Chrysosporium linfenense*. A. Conidiogenous structures and mature conidia B. Racquet hyphae C. A growth of *C. linfenense* along hair on Czapek agar medium with carbon-free and nitrogen-free sources D. A hair on Czapek agar medium with carbon-free and nitrogen-free sources without inoculation C1. The hair degraded by *C. linfenense* after 14d(×400) D1. The hair without being degraded (×400)(Bars=10 μm).

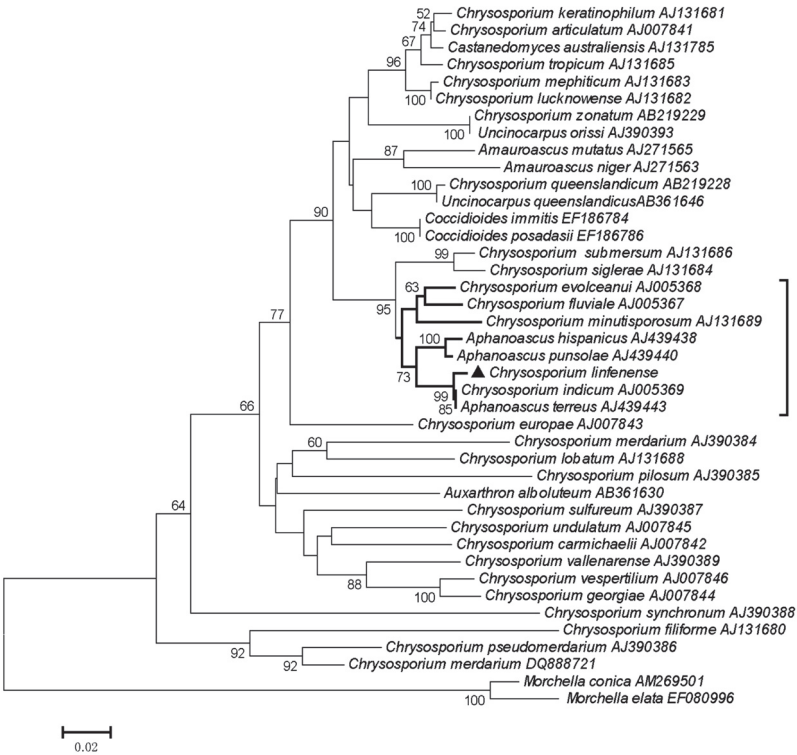

Fig. 2 Phylogenetic tree based on analysis of ITS1-5.8 S rDNA -ITS2 sequences of *C. linfenense* and some related species.

From the phylogenetic tree, *Chrysosporium linfenense*, *C. evolceanui* (H.S. Randhawa & R.S. Sandhu) Garg 1966 (= *C. pannicola* (Corda) Oorschot & Stalpers; Oorschot 1980), *C. fluviale*, *C. indicum, C. minutisporosum*, *Aphanoascus hispanicus* Cano & Guarro 1990, *A. punsolae* Cano & Guarro 1990, and *A. terreus* (H.S. Randhawa & R.S. Sandhu) Apinis 1968 were grouped in a subclade. In this clade, *C. evolceanui*, *C. fluviale,* and *C. minutisporosum* differed from *C. linfenense* in their morphological characters (see Table 2).

Five other related species were supported (73%) on the adjacent subclade with two branches. One branch (100% support) included *Aphanoascus hispanicus* and *Ap. punsolae*; the second branch (99 % support) included *C. linfenense*, *Aphanoascus terreus,* and *C. indicum*, suggesting a close phylogenetic relationship. As early as 1968, Apinis A.E. reported that *C. indicum* is an anamorph of *Aphanoascus terreus.*

Although *C. linfenense* and *C. indicum* were grouped on the same branch, their genetic separation was also supported (Fig. 2). The morphological

differences between *C. linfenense* and *C. indicum* are likewise marked (see Table 2). Thus, both morphological and molecular analyses support strain GZUIFR-H31 as a new member of *Chrysosporium*.

Keratinolytic activity

Human hair was inoculated by *C. linfenense* on Czapek agar medium with carbon-free and nitrogen-free sources, which was incubated at 30°C for 14d. The *C. linfenense* hyphae grew densely along the hair, which was obviously degraded after 14 days (Fig. 1, C–C1). This keratin degradation shows that *Chrysosporium linfenense* GZUIFR-H31 is a potential keratinase-producing strain.

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